VIII.—*On the Relations between certain Diatoms and the Fission-products of a Parasitic Alga* (Chlorochytrium). By H. Charlton Bastian, M.A., M.D., F.R.S., F.L.S.

[Plate XV.]

Much interest was excited in 1872 owing to the discovery by F. Cohn * of an alga existing as a parasite in the thallus of the ivy-leaf duckweed (*Lemna trisulca*). This was followed in 1877 by the discovery of another parasitic alga by Prof. Perceval Wright † infesting various marine algæ. Since this time several other forms have been discovered, and rather an extensive literature has grown up concerning *Chlorochytrium* and allied genera. A key to some of this literature will be found in de Tonis's 'Sylloge Algarum,' vol. i. (1889) p. 636, in which an attempt was made to classify the various species then known.

Among the new forms there is one, *Ch. Knyanum*, found in *Lemna gibba* and in *L. minor*, which was examined and figured by G. Klebs ‡ in 1881. This is evidently the alga that I have of late met with very abundantly in both these species of duckweed, and to which my present remarks will refer.

I find during autumn and winter among duckweed from various localities many dead and decolorized leaves, having a greyish-white and somewhat gelatinous appearance. Such leaves may be easily picked out by spreading some of the duckweed in a thin stratum of water over a white dish. It will be found that the decolorized leaves are all devoid of rootlets, and possibly this loss of the rootlets may have been the main cause leading to the premature death and change in the appearance of the leaves.

Examination with a hand-lens, magnifying eight or ten diameters, will show in many of such leaves that the upper greyish-white surface is flecked with minute specks of an emerald-green colour, sometimes abundantly and sometimes sparsely, while examination of these or other leaves under the microscope will often show an abundance of the early stages of such bright green specks, so minute as to have been invisible with the mere hand-lens.

It is best to pick out the smaller leaves for microscopical

* 'Beitrage zur Biologie der Pflanzen,' Heft xi. p. 87.
† Trans. Roy. Irish Acad. vol. xxvi. p. 13.
‡ Botan. Zeitung, 1881, p. 248, t. iii. figs. 11–15.

examination, and even then (especially with *L. gibba*) the examination can often only be satisfactorily carried out by placing one of the leaves in water on an excavated glass slip (taking care that its upper surface is uppermost), and gently compressing the leaf, if necessary, with the cover-glass.

An examination of a very large number of these infected leaves has enabled me to ascertain the following facts:—

The very active spores of the *Chlorochytrium* penetrate to some of the intercellular spaces of the leaf through the stomata. Single spores or such bodies after a primary fission may be seen just within the stomata (Pl. XV. fig. 1, A). Sometimes the entire spore or the segments of the once or twice divided spore will grow considerably before undergoing any further fission (as in B), though more commonly division goes on so as to produce eight or more cells (C), which, as they grow, soon become tightly packed within the now dilated substomatal space (D). Examination of the surface of the leaf over one of these patches will always reveal a stoma greatly dilated and almost circular in shape *.

The mode of infection in *L. minor* and *L. gibba* is therefore altogether different from that described by Cohn as occurring in *L. trisulca*. In that species of duckweed there is, curiously enough, an absence of stomata. The average shape and appearance of the patches of *Chlorochytrium* in *L. trisulca* is also rather different from that of the patches in the other two duckweeds, and the latter patches also lack the distinct and often thick bounding membrane which occurs round the patches in *L. trisulca*.

In each of the forms the tendency is to an ultimate production of minute spherical or ovoidal zoospores, which, after exhibiting a swarming movement, may make their way out of the space in which they have been developed. It often happens, however, in each of these forms of *Chlorochytrium* that the zoospores may, either in whole or in part, not succeed in escaping, but come to rest within their respective cells or spaces (fig. 2, B, × 375).

What I have further to say refers especially to *Ch. Knyanum*, and to this form as it occurs in *L. gibba*.

In some of the smaller patches composed only of two or of four enlarged cells it may occasionally be seen that segmentation of the contents of one of the cells only has occurred, while others have remained unaltered. This same kind of independence in the life of the cells occurs also in larger

* All the components of figs. 1, 2, and 3 have been magnified 250 diameters except fig. 2, B; this latter, as well as all the components of figs. 4, 5, and 6, have been taken at a magnification of 375 diameters.

aggregates, some of the individual units of which may often be seen entire and undivided, while the contents of others are in different stages of fission down to the final stage of spore-formation.

It seems probable that sometimes the swarm-spores are formed by a simultaneous segmentation of the cell-contents into the brood of spores, but in other cases, as was clearly shown by G. Klebs* for *Ch. Lemnæ*, the cells undergo successive processes of fission till the swarm-spores are produced. This latter kind of process I have found to occur very abundantly in *Ch. Knyanum*.

Multitudes of partially empty spaces may be seen containing large or small specimens of these intermediate fission-products, those within the same space being either all of one size (fig. 2, C) or of very different sizes. Other spaces may be seen still full and distended with *Chlorochytrium*, the constituent cells of which exhibit very different degrees of segmentation (fig. 2, A). Some have become resolved into the minute zoospores (fig. 2, B), while others have remained as fission-products varying much in size. Some writers have spoken of some of the larger forms as being probably "resting-spores."

It seems to me, however, that it can only with certainty be said that the *Chlorochytrium* cells undergo processes of division to a variable extent, so as to yield fission-products of very different sizes, and that, presumably under the influence of some unfavourable conditions in their environment, some of the products at each of these stages may undergo no further changes of a normal kind, and thus may never give rise to *Chlorochytrium* spores.

This brings me to one of the important points which this communication is destined to make known, which is, that in the later stages of the life of *Ch. Knyanum* the fission-products within the intercellular spaces of the leaf are often found to be more or less intermixed with diatoms, varying not a little in size and in shape.

This association is met with sometimes in spaces none of the contents of which have escaped, and then the contrast is great between the beautiful emerald-green of the algoid cells and the brownish-yellow colour of the diatoms mixed therewith. At other times partially empty spaces are seen containing the fission-products of the alga alone (Pl. XV. fig. 2, C), diatoms alone (fig. 2, D), or a mixture of the two kinds of units (fig. 3, A, B, C, D).

* *Loc. cit.* Taf. iii. figs. 10, *a, b, c.*

More rarely spaces are found densely packed with brownish-yellow diatoms only, in different stages of growth and development, except perhaps for the association of one or two minute algoid corpuscles (fig. 4, A, B, C, D) *.

In regard to the diatoms themselves, these are sometimes very small and rudimentary (as in fig. 3, A, and in the upper part of D), but at others they are much larger (as in fig. 3, B, C, D). These larger sizes are either fairly broad and ovoid, like *Naviculæ*, or else narrow and elongated, like *Nitzschiæ* (fig. 4, C, D).

In almost all cases, however, the diatoms have the appearance of being immature; they have ill-developed siliceous envelopes and are all quite full of brownish-yellow endochrome. There are also at times indications that growth and multiplication of these immature forms is or has been taking place, looking to the way in which they are occasionally ranged side by side in short rows in some of the half-empty spaces (as in fig. 3, A, and in the upper part of D).

The substomatal spaces which have been tenanted by the *Chlorochytrium* are characterized, as I have said, by a greatly distended and almost circular stoma, and often by having their walls stained of a more or less distinct rust-colour. Indications of the latter change are to be seen in fig. 2, C, D †. It is a fact of much importance that diatoms are never to be found in any of the substomatal spaces except in those which either actually contain or bear marks of having been previously tenanted by *Chlorochytrium*.

Unfortunately I have found it very difficult to photograph some of the most remarkable specimens I have met with. This has been due to a combination of causes. It has been partly owing to the light having to pass through the whole thickness of the leaf, partly because of the staining of the walls of the spaces, partly because the photograph yields no discrimination in shade between the emerald-green colour of the alga and the characteristic brownish yellow of the diatom, and at other times owing to the diatoms being so densely packed within their little subepidermal pockets that their individual forms cannot be clearly shown, as in fig. 4, in which two of the spaces (C and D) were closely packed with

* The specimens shown in this figure looked much more densely packed before the leaves from which they were taken were immersed in glycerine in order to facilitate the taking of the photographs. All the other photographs have been taken from leaves immersed in water only, except fig. 6, A, which was also taken from a specimen in glycerine.

† Of course these two characteristics, belonging to different planes, can never be seen together in the same photograph.

small but long and slender diatoms like *Nitzschiæ,* while fig. 3, C, D, contained broader and more ovoid organisms of the *Navicula* type.

It occasionally happens that the spores of the *Chlorochytrium* force their way from a closely packed space in which they have been produced, whence exit is not easy, in between various of the contiguous sub-epidermal cells, and occasionally in these situations I have also found diatoms. Spores in these situations between the spherical cells are shown on the right side of fig. 1, D.

There is another point of much interest to be mentioned.

Sometimes one of the epidermal cells, of zigzag outline, will here and there be found filled by a light green alga having the appearance of being a species of *Chlorochytrium* (fig. 5, A). Other of these cells may be found in which such bodies seem about to undergo fission into several smaller cells (fig. 5, B), and others still in which the original cell has divided into small green ovoid products (C) or into a number of more minute zoospores. In one case such zoospores were seen to have assumed a yellow colour and some of them seemed to be elongating, as was the case with some of the segments shown in fig. 5, D. Many other of these isolated epidermal cells have been found containing either small ovoid diatoms only (fig. 5, F) or a mixture of such diatoms with green fission-products as in fig. 5, E—just as I have found the two kinds of bodies associated in the much larger substomatal spaces.

The diatoms in the epidermal cells are always small, commonly of about the same size, but not invariably so, and mostly having the appearance of being minute *Naviculæ.*

How the *Chlorochytrium* spores obtain an entry into the epidermal cells I am unable to state; but being actively motile, it would clearly be much easier for them to get in than for the diatoms to do so.

It seems most probable that it is the spores of *Ch. Knyanum* which infect these epidermal cells, and it seems possible that they may penetrate them from a substomatal space, as I have often, though by no means invariably, found such infected epidermal cells just over, or by the side of, one of these spaces.

What interpretation is to be given concerning the association of the diatoms with the *Chlorochytrium* fission-products?

Only two possibilities seem to present themselves:—

(*a*) The diatoms have, like the algæ, obtained entry to the subepidermal spaces through the stomata.

(*b*) The diatoms have been produced *in situ* by a transformation of the fission-products of the alga.

The first of these possibilities it will be convenient to speak of as the Infection Hypothesis and the second as the Transformation Hypothesis.

(a) *Infection Hypothesis.*—The difficulty in accounting for the facts seems to me to be extreme in accordance with this supposition, especially if we bear in mind what is authoritatively known concerning diatoms. The important points are these:—

1. No *motile* spores are known, and previous to 1896 there was no certain knowledge concerning the existence of spores of any kind in diatoms. The important discovery by George Murray of undoubted spores or germs, originating by a process of rejuvenescence, in species belonging to three marine genera * constitutes all that is certainly known at present on this subject.

2. It is commonly stated by writers that individual diatoms do not increase in size †, increase in bulk of diatoms being only brought about as a result of "conjugation," which is admitted to be a comparatively rare process.

3. Previous to the above-mentioned discovery by George Murray diatoms were said to be formed only (*a*) by a process of "conjugation" or rejuvenescence, or (*b*) by fission, which is the common process, and one that involves a very slight diminution in size of the products ‡.

Such facts concerning diatoms in general must be borne in mind in conjunction with these others more specially bearing upon the question now under consideration.

4. The substomatal spaces which either are or have been tenanted by *Chlorochytrium* probably constitute much less than 10 per cent. of those existing on most leaves of the duckweed, yet no diatoms are ever to be seen in the other 90 per cent. of the substomatal spaces.

5. The purposeless to-and-fro movements of some diatoms when free in a fluid, and their absence of movement when lying on the surface of a leaf, seem quite incompatible with the notion of their selective penetration through certain special stomata only.

6. A point of still greater importance is the fact that

* Proc. Roy. Soc. Edinb. 1896–97, p. 207.

† Wolle, 'Diatomaceæ of North America,' 1890, p. 11; Smith's 'British Diatomaceæ," vol. i. 1853, p. xxiv, and 1856, vol. ii. p. vii; and Pritchard, 4th ed. 1861, pp. 58, 61–63.

‡ Wolle, *loc. cit.* p. 11.

diatoms are never to be seen in the spaces in which the *Chlorochytrium* is in one of its early stages of development; they are to be found only in association with its later stages, where some of the final segmentations have been taking place, and often where the patches are so old that the walls of the spaces containing them are stained of a rust-colour.

7. None of the diatoms found either within the spaces or within their ramifications between surrounding cells have ever been seen to move.

8. Moreover, where the diatoms exist they are often intimately intermixed with the algoid cells; they are also to be seen in the peripheral regions of spaces, even when these are still full, and small specimens are likewise to be found between the spherical subepidermal cells contiguous to the invaded space. Such facts are incompatible with an entry of diatoms from without, especially if we bear in mind what has been said under the last two heads.

9. Again, where the diatoms exist they not only vary much in size and shape in different spaces, but even within different regions of the same space.

Taken as a whole these various facts seem to me absolutely to negative the Infection Hypothesis as a means of accounting for the association of the diatoms with the fission-products of *Chlorochytrium* in the subepidermal spaces.

(b) *Transformation Hypothesis.*—The facts which are so incompatible with the foregoing hypothesis will be found either to offer no difficulties to, or to be capable of receiving a ready explanation in accordance with, the transformation hypothesis. This hypothesis is also strengthened by other facts not previously referred to.

1. The absence of the diatoms from the 90 per cent. of the substomatal spaces which are not infected by the algæ is explained.

2. The absence of movements on the part of the diatoms in question affords no difficulty.

3. The absence of the diatoms from the *Chlorochytrium* spaces during the early stages of the development of the alga affords no difficulty and is explained.

4. The variation in the size of the diatoms is explained, in the main, by the varying size of the fission-products of the alga. The two kinds of units very commonly coexist, and where the algoid cells are small the diatoms are small, where they are of medium or larger size the diatoms are similarly of medium or larger size. Such variations in the size of the algoid cells are very common within the same infected space,

and then, when diatoms are present, they are also of various sizes.

5. Old, partially empty, spaces are often to be seen containing the *Chlorochytrium* fission-products, small or large; others may be found containing diatoms, small or large; and others again partially empty, but containing a mixture of the algoid fission-products with diatoms of a corresponding size.

6. Other spaces still densely filled will show, with the algoid cells, diatoms either packed in their midst or occupying the boundaries of the spaces, and often differing greatly in size in the two situations. They are likewise to be found occasionally in the narrow spaces between contiguous spherical cells, where, as I have said, algoid spores from the parent brood not unfrequently penetrate.

7. In the spaces where the algoid cells and the diatoms are mixed some of the cells may be seen to have assumed the brownish-yellow colour of the diatoms, and some of such cells may also be seen more or less elongated and apparently developing into diatoms.

8. The majority of the diatoms have an immature appearance. The siliceous envelope in the great majority of them seems to be either absent or very imperfectly developed, and unmistakable evidence that multiplication of these immature diatoms has taken place is frequently to be seen *.

There is no probability, and no one, I think, is likely to maintain, that diatoms are normal phases in the life-history of this parasitic alga; and as a careful consideration of the evidence as a whole appears utterly irreconcilable with the infection hypothesis, we seem unavoidably driven to the conclusion, which is so congruous with all the facts, that the diatoms in question are heterogenetic products actually produced by the transformation of the cells of the alga, alike in the substomatal spaces and in the epidermal cells.

I include the epidermal cells in this statement, because

* Some of the differences in size, apart from differences in the size of the algoid fission-products from which the diatoms have originated, may be due to increase in bulk of these immature organisms. Although this is at variance with commonly received views, it is in accord with the observations of George Murray, who says (*loc. cit.* p. 216) that young diatoms formed within a parent by a process of rejuvenescence when liberated by "the separation of the parent valves at the girdle may grow, divide, and multiply before fully attaining the characteristic external sculpturing and adornment of the parent." Young diatoms originating in fresh water may find silica in all pond-water. The ammonia contained in rain-water, like other alkalies, easily dissolves silica or aluminium silicate when in a finely pulverised state, and one or other of these compounds is to be found in all soils (see Prof. Edwards, "On the Solubility of Silica," 'The Chemical News,' Jan. 1896, p. 13).

almost all that has been said against the infection hypothesis and in favour of the transformation hypothesis as accounting for the presence of the diatoms in the substomatal spaces holds good also in regard to their presence in the epidermal cells. In one respect the argument is even stronger in its application to them, since there is much evidence to show that diatoms are only found in those epidermal cells which are or have been tenanted by the alga, and such infected cells never constitute more than the smallest fraction per cent. of those existing on the whole upper surface of a leaf.

A further point of extreme importance is to be found in the very great differences in the size and shape of the diatoms, according as they originate from the small or the larger algoid fission-products. Yet these variations, for which no other contributory cause is apparent to us, are so great that botanists unaware of the origin of the diatoms, and finding them in the *Chlorochytrium* spaces, would almost certainly regard some of them as belonging to different species of the same genus, and others even as representatives of distinct genera. This, however, is a subject which must be left for future investigation.

It was suggested to me by a distinguished botanist, to whom I showed some of the specimens of duckweed containing in their substomatal spaces and epithelial cells mixtures in various proportions of *Chlorochytrium* segments and diatoms, that their association might be explained by the Infection Hypothesis, backed by the assumption that *Chemotaxis* had been in operation, which in this case would mean that the physico-chemical processes associated with the growth and multiplication of the algæ within the spaces were capable of giving rise to products exercising an attractive influence upon the diatoms.

It was not pretended that there was any direct evidence in favour of this assumption; it was advanced as a possible explanation and merely to stave off the conclusion, otherwise inevitable, that the diatoms had been produced by the transformation of the cells of the alga.

A careful and unbiassed consideration of the following facts will, however, I think make it plain that the evidence is overwhelmingly against Chemotaxis and the Infection Hypothesis:—

1. Chemotaxis can only be supposed to operate at short distances; but such diatoms as are found within the spaces are never to be seen on the surface of the duckweed.

2. The diatoms that are commonly met with on the surface

of the thallus (a comparatively large *Navicula* and a *Cocconeis*) are never found within the substomatal spaces or the epithelial cells.

3. Chemotaxis implies a direct power of movement in response to an attractive influence; but none of the diatoms on the surface of the duckweed within the spaces or within the epithelial cells have ever been seen to move.

4. The diatoms in the spaces are found intimately intermixed with the algoid cells, and generally in situations to which they could not be supposed to have the power of penetrating.

5. The diatoms can often be seen to have replaced algoid cells rather than to have pushed them aside.

6. Finally, in places, the algoid cells can be seen elongating into the forms of the diatoms and at the same time changing from a bright green to a yellowish-brown colour.

Moreover, since making these observations on *L. gibba* and *L. minor* I have ascertained that similar transformations of some of the fission-products of the *Chlorochytrium* which infests *L. trisulca* are also to be met with in that species of duckweed. The diatoms found in this species have been almost always very small and of the *Navicula* type—no *Nitzschiæ* having ever been seen in association with the segmentation-products of this particular variety of *Chlorochytrium*, although the duckweed bearing it has been taken from one of the same ponds from which I have obtained my supplies of *L. gibba* and *L. minor*. In fig. 6 (× 375) some of the combinations that have been met with are shown. In A four small spaces are shown: in the upper one algoid segments and diatoms were intermixed; in the one on the left young diatoms were seen forming, the contents of this space being distinctly paler than those of the other two spaces, in which the diatoms were more fully formed and more closely packed. In B two or three fused contiguous spaces are shown in which algoid cells and diatoms, together with various intermediate forms, were intimately intermixed; while C is the only space that I have yet found in *L. trisulca* containing diatoms as large as are there represented. They were mixed with *Chlorochytrium* cells and other minute diatoms, though the latter are not recognizable in the photograph.

It is worthy of note that in *Lemna trisulca* there are no stomata. The active algoid spores penetrate, as F. Cohn showed, by boring between the epithelial cells into subjacent spaces, where they increase and multiply in practically closed cavities and become also surrounded by a kind of capsule. Subsequently their active spores make their way out through very minute apertures which they themselves form; but in this species of duckweed there are no widely dilated stomata through which in earlier or in later stages, should they attempt it, diatoms would be free to enter.

Another point is also of much importance, and that is the frequency with which diatoms may be seen around the periphery of spaces still densely crowded with *Chlorochytrium*-products which have not yet begun to emerge. These diatoms therefore make their appearance within closed cavities and often in regions far removed from the original point of entry of the active algoid spore. No infection hypothesis, even backed by a further hypothesis of chemotaxis, is, I submit, capable of explaining the presence of these diatoms. They are evidently formed where they are found by a transformation of the algoid cells, and different stages of the process may often be clearly recognized, the spherical cells, as I have said, becoming elongated and changing from a bright green to a brownish-yellow colour as they take on the forms of the diatoms *.

EXPLANATION OF PLATE XV.

All the components of figs. 1–3 have been photographed at a magnification of 250 diameters except fig. 2, B; this, as well as all the components of figs. 4, 5, 6, have been taken at a magnification of 375 diameters.

Fig. 1. A, shows two stomata of *Lemna gibba* which have been penetrated by spores of *Chlorochytrium Knyanum*; in the lower one the spore has divided. B, a group of *Chlorochytrium* cells within a substomatal space. C, another group (older) in which the cells are more closely pressed together. D, a still older patch of *Chlorochytrium* that has begun to yield spores, some of which have passed out and are lodged between the spherical cells situated underneath the epidermis.

Fig. 2. A, several contiguous patches of *Chlorochytrium,* showing fission-products varying much in size. B, a single large *Chlorochytrium* cell which is full of spores. C, a partially empty sub-stomatal space containing fission-products of medium size. D, a similar partly empty space containing diatoms of medium size. The wall of the space was distinctly more brown in D than in C.

Fig. 3. A, a mixture of fission-products and of diatoms of different sizes within a space. B, another space containing rather larger fission-products mixed with rather large diatoms. C, like the last, only with a much larger number of diatoms than of fission-products. D, a space containing larger diatoms still, together with some very minute ones in an upper extension of the space.

The diatoms shown in this figure are probably different kinds of *Naviculæ.*

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* In my 'Studies in Heterogenesis,' Part iii. Sec. xiii. p. 181, I have described and illustrated the origin of diatoms from other algoid cells which are often to be found on the surface and in the substomatal spaces of the different species of duckweed.

Fig. 4. A and B, two spaces containing a mixture of small *Chlorochytrium* segments and of developing diatoms. C and D, two other spaces with very few green fission-products, but with many much elongated diatoms of the *Nitzschia* type.

Fig. 5. A, an epidermal cell full of *Chlorochytrium.* B, two other cells containing *Chlorochytrium* which seems about to divide into fission-products. C, another epidermal cell containing *Chlorochytrium* which has divided into several nearly equal segments. D, an epidermal cell containing many small segments which had assumed a yellowish colour and were beginning to elongate. E, an epidermal cell containing diatoms mixed with some green fission-products. F, another epidermal cell containing a number of diatoms, but only two green *Chlorochytrium* fission-products. All these diatoms resembled small *Naviculæ*.

Fig. 6 shows diatoms developing from the fission-products of *Chlorochytrium Lemnæ*. A, four contiguous spaces, of which the upper one contained a mixture of minute fission-products and of diatoms, that on the left diatoms in an early stage of development, while the other two were densely packed with more mature *Naviculæ*. B, two large contiguous spaces containing an intimate mixture of fission-products and of developing diatoms. C, another space containing some fission-products and a number of diatoms larger than are usually to be found within the sub-epidermal spaces of the ivy-leaf duckweed.

IX.—*Descriptions of Three new Batrachians from Tonkin.* By G. A. Boulenger, F.R.S.

A collection made by Mr. H. Fruhstorfer, of Berlin, in the Man-Son Mountains, Tonkin, altitude 3000–4000 feet, and purchased from him by the Trustees of the British Museum, contains, in addition to several little-known frogs (*Leptobrachium carinense*, Blgr., *L. pelodytoides*, Blgr., *Hyla simplex*, Bttgr., *Rana Guentheri*, Blgr., *R. graminea*, Blgr., *R. nigrovittata*, Blyth, *R. Ricketti*, Blgr., *Oxyglossus Martensii*, Peters, *Rhacophorus verrucosus*, Blgr.), examples of three new species, one of which is even entitled to be regarded as the type of a new genus.

Ophryophryne, gen. nov.

Pupil horizontal. Mouth small, toothless, inferior. Tongue pear-shaped, adherent, entire, swollen and cup-shaped behind. Tympanum distinct. Fingers free, toes nearly free, the tips not dilated. Outer metatarsals united. Omosternum cartilaginous ; sternum with a slender bony style. Sacral vertebra with very strongly dilated diapophyses and one condyle for articulation with the coccyx.

Like *Cophophryne*, Blgr., this genus presents an interesting